WADC TECHNICAL REPORT 52-222

THE EFFECTS OF DRUG-INDUCED ALTERATIONS OF THE AUTONOMIC NERVOUS SYSTEM ON THE RESPONSES OF MAMMALS TO OXYGEN DEPRIVATION

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FOREWORD

This report was prepared under USAF Contract No. W 33(038) ac-18469. The original contract dated 14 September 1947 was initiated under AFP 47844, and sponsored by the Aero Medical Laboratory, Directorate of Research, Wright Air Development Center, under the research and development project identified by RDO No. 696-61, High Altitude Physiology, with Dr. John W. Wilson acting as project engineer.

The actual experimental work described herein was completed late in 1949. Part of the work was released as a Memorandum Report dated 10 February 1949, No. MCREXD-696-79H of the Air Materiel Command. The individual foci of experimentation were written up, approved by the Air Materiel Command and published in the Proceedings of the Society for Experimental Biology and Medicine, the American Journal of Physiology and the Journal of Aviation Medicine. Two units remain unpublished.

ABSTRACT

The cardiovascular reactions of the mammal to sudden withdrawal of oxygen from the inspired atmosphere include rise of blood pressure and rise of heart rate. While reflexes activating the sympathetic division of the autonomic nervous system are involved in creating these responses, the importance of epinephrine release and of neural activation of arterial musculature in specific areas remain unassessed.

With the discovery in recent years of new chemical agents which present with varying specificity abilities to block the autonomic effector paths, the possibility beckons that use of these drugs may elicit basic information about the reaction to oxygen want.

Accordingly several hundred experiments were performed in anesthetized dogs and rabbits. Clarification of the mechanisms did not result but certain items of basic information gleaned have been reproduced here in the form of their scientific publication. These items may be inventoried briefly as follows:

The quantitative mean arterial pressure response of the anesthetized dog to carotid occlusion and to hypoxia proved to be direct functions of pre-existing mean arterial pressure. In the case of carotid occlusion the relation ceased at approximately 60 mm Hg pressure. Specific interference with this relationship by drugs was demonstrated.

The effect of anesthesia on ventilation volume was shown to be a profound rise under urethane and a fall under pentobarbital. The hyperventilation induced by hypoxia was much greater under the former than under the latter anesthetic.

The anesthetized dog and the unanesthetized rabbit were deprived of their normal resistance to abrupt oxygen-want by drugs which reversed the pressor action of epinephrine. The lack of resistance was manifested by hypotensive response to oxygen-want and by early failure to maintain ventilation.

The resistance of unanesthetized animals to the less abrupt oxygen-want of chamber decompression was shown to be only slightly to moderately impaired by adrenergic or by cholinesterase blockade. In this study it was shown that the methods of treating quantal data developed by the pharmacologists were applicable to the data obtained in chamber decompression experiments.

PUBLICATION REVIEW

This report has been reviewed and is approved.

FOR THE COMMANDING GENERAL:

Robert H. Blount

ROBERT H. BLOUNT
Colonel, USAF (MC)
Chief, Aero Medical Laboratory
Directorate of Research

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SECTION I

Respiratory Arrest in Rabbits Exposed to Hypoxia after Dibenamine.

EBEN DUSTIN AND GEORGE MAISON.

The observations here reported were made as part of a systematic elucidation of the effect of alteration of function of the autonomic nervous system on the response of the intact animal to hypoxia.

Rabbits underwent early and sudden respiratory arrest on exposure to hypoxia after receiving Dibenamine (N, N-dibenzyl-β-chloroethylamine) in ethyl alcohol and propylene glycol aa by vein. Control animals underwent such arrest very rarely even under more prolonged similar exposure to hypoxia.

Procedure. Normal rabbits (weight 1.5 to 4 kg) under intravenous pentothal anesthesia were subjected to tracheotomy. Procaine hydrochloride 2% was infiltrated through the margins of the incision and skin closure around the cannula was made with skin clips.

The rabbits were restrained in the supine, head low position on a table offering a slope of 15° from the horizontal in order to aid the venous return. After recovery (minimum 30 min.) from the general anesthesia the inspiratory ventilation volume on room air was measured by spirometer. This was made possible by an hydraulic flutter valve‡ interposed between the tracheal cannula and the spirometer. The dead space of the system was 3 to 5 cc. This compares favorably with the dead space of the eliminated respiratory tract above the cannula in the rabbit.

Measurements were then continued as the animal was abruptly shifted to an atmosphere of 5% oxygen in helium for a period of 5 minutes. After one or a series of such control

‡ A modification of the type pictured on page 206, Jackson, D. E., *Experimental Pharmacology and Materia Medica*, 2nd Edition, 1939, C. V. Mosby Co., St. Louis, Mo.

TABLE I.
Occurrence of Respiratory Arrest on Exposure to *Circa* 5% O_2 for 5 Minutes.

Treatment	No. of rabbits showing no arrest	No. of rabbits showing arrest
Normal	49	2
Dibenamine in Propylene Glycol and Ethyl Alcohol āā 12-24 mg/kg*	2	20
Dibenamine in 50% Alcohol 12-24 mg/kg	1	4
Dibenamine in 50% Propylene Glycol 12-24 mg/kg	1	5
Propylene Glycol 50% 2 cc	6	0
Alcohol 50% average 0.5 cc	6	0
Propylene Glycol and Absolute Alcohol āā average 0.5 cc	6	0

* By Chi square test the probability of chance distribution of 29 positive in 33 as opposed to 2 positive in 49 is less than 1 in 10,000.

observations on the effect of hypoxia, Dibenamine (at a concentration of 50 mg/cc in the chosen medium)§ was administered in marginal vein of ear in calculated dose over a period of one minute. Usually the primary dosage of Dibenamine was 12 mg/kg.

After a variable delay the ventilation volume on room air was measured and the animal was again presented with the hypoxic atmosphere for 5 minutes. If respiratory arrest occurred artificial ventilation was established after 1 to 2 minutes of apnea, by means of human expired air through the tracheal cannula. Successive periods of hypoxia were rarely at intervals less than 20 minutes.

Gas mixtures were made in large rubber bag of some 1500 liter capacity and analyzed for oxygen content repeatedly through the day's experiments. Maximum variation of different days' mixtures was 4.0-5.5% oxygen (mean $5\% \pm 0.5$).

Observations. Table I records the incidence of respiratory arrest in rabbits exposed to hypoxia for 5-minute periods after various medications.

Normal Controls. The 2 cases of respiratory arrest in 49 normals showed arrest only once each in several trials. Time required for arrest to occur was 230 and 240 seconds respectively. It was repeatedly shown that 5-minute periods of hypoxia did not predispose to respiratory arrest in later 5-minute periods of exposure (11 animals up to 9 trials each over periods up to 6 hours).

Dibenamine: Incidence. In the total series of 38 animals given Dibenamine 5 died within 10 minutes of the intravenous injection of 12 mg/kg. Twenty-three animals showed arrest during hypoxia after the first 12 mg of the drug. Six animals required a second dose at the end of an hour or more before arrest would result from hypoxia. The remaining 4 animals never showed respiratory arrest.

The pattern of respiratory arrest after Dibenamine was bizarre. The rise of ventilation volume at the beginning of the period of hypoxia (average 59% rise) differed little from that of the control hypoxic condition (average 72% rise). However, after an average exposure of 158 seconds (in 44 trials, range 30 to 300 seconds) the respiration became more labored, followed abruptly by an apparently tonic contraction of the diaphragm, accompanied by twitching movements of the abdomen. Simultaneously the tidal volume fell to zero and the tonic contraction faded out. Thereafter no respiratory movements appeared in most animals though one or two gave a feeble gasp reflex.

Duration of susceptibility to respiratory arrest. In animals tested serially to determine the time course of susceptibility to respiratory arrest on exposure to hypoxia, arrest could be elicited at the earliest in most animals between 20 and 40 minutes (mean 33 minutes) after the administration of the drug. Susceptibility endured throughout the period of test (up to 4 hours) in a number of animals. In others the ability to compensate for hypoxia returned, after as little as

§ Media used included Propylene glycol and absolute alcohol āā most commonly, Propylene glycol and water āā or absolute alcohol and water āā on other occasions.

48 minutes following administration of Dibenamine.

Controls receiving solvents without Dibenamine. See Table I.

Discussion. That the susceptibility to respiratory arrest is not dependent on some residual effect of the pentothal was demonstrated by the occurrence of respiratory arrest in cats under the same conditions except that cyclopropane was utilized during the preparatory period. The pilot experiments in the cat also showed that the susceptibility to arrest is not species specific in the rabbit alone.

That the helium was not an active factor in the elicitation of respiratory arrest was shown in animals tested on nitrogen and oxygen (5%). Five minutes on this mixture never caused arrest in several trials in each of 11 animals tested before Dibenamine. It was at least as potent in producing respiratory arrest after Dibenamine.

That the phenomenon observed was a true respiratory arrest was shown by the demise of 8 animals allowed to go untreated in the apnea which occurred during hypoxia after Dibenamine. No animal ever recovered spontaneously from this state of arrest. On resuscitation spontaneous respiration was reestablished promptly after a minute or more of artificial ventilation with human expired air.

As to the mechanism of action of Dibenamine in inducing respiratory arrest during hypoxia little evidence is available. Pilot experiments have shown that bilateral vagotomy, with its removal of inhibitory sensory discharge does not prevent the occurrence of arrest on hypoxia after Dibenamine (3 animals). Three experiments suggested that Dibenamine does reverse the normal pressor response to hypoxia in rabbits as in other species. However, the fall of blood pressure is inconstant and respiratory arrest has been noted unaccompanied by a depressor response. Acapnia seems an unlikely cause of the apnea, since the hyperventilation normally seen in hypoxia of this grade is at least as great as that after Dibenamine. Finally, a direct depressant action of Dibenamine on the respiratory center seems unlikely, since the center still responds to the carotid body under hypoxia by inducing hyperventilation.

Summary. Rabbits given Dibenamine 12 to 24 mg/kg in propylene glycol or ethyl alcohol by vein show sudden respiratory arrest within 3 minutes of exposure to hypoxia (circa 5% O_2 in helium).

SECTION II

CAROTID-OCCLUSION-PRESSOR REFLEX: INFLUENCE OF EXISTING MEAN ARTERIAL PRESSURE, OF ANESTHETICS AND OF GANGLIONIC- AND ADRENERGIC-BLOCKING DRUGS

GEORGE PROCHNIK, GEORGE L. MAISON AND J. W. STUTZMAN

THE effector limb of the reflex arc involved in the pressor response to carotid occlusion is generally agreed to be in the sympathetic division of the autonomic nervous system. In attempting to utilize this reflex response as an index of the functional state of the sympathetics it became necessary to know the relation between existing blood pressure and magnitude of response. Review of the literature showed that this relation was well established for distension of the isolated carotid sinus (1) but not for carotid occlusion. Specifically it was desired to determine how carotid-occlusion-pressor responses should be reported. Experimental validation of the usual practice of reporting responses as percentage of initial mean arterial pressure was not found. The effect of common laboratory anesthetics on the response was ill-defined. While certain authors state that tetraethylammonium chloride (TEA) (2, 3) and adrenergic blocking drugs (3–8) reduce or obliterate the response, quantitative data were not found.

The experiments described below showed that there was a variable but direct proportionality between pressor effect of carotid occlusion and pre-existing mean arterial pressure; that ether, pentobarbital and urethane individually as anesthetics did not apparently vary the foregoing relationship; that the relationship was greatly altered and the pressor response greatly reduced by doses of TEA (adequate to block response to peripheral vagus stimulation), by doses of dihydroergocornine (DHO 180, allegedly a centrally as well as peripherally acting adrenergic-blocking drug) inadequate to reverse completely epinephrine, and by epinephrine reversing doses of benzyl bisβchloroethyl amine (BBB).

METHOD

Dogs were anesthetized with pentobarbital (*ca.* 35 mg/kg.) or urethane (*ca.* 1.5 gm/kg.) administered intravenously or with ether by inhalation (drop-induction followed by Wolff bottle).

Mean arterial pressure was recorded by mercury manometer from the femoral artery. Record-

ings were made on Teledeltos paper on a drum moving approximately 10 mm/minute. Drugs were infused via a venous cannula in the femoral or jugular vein at a constant rate. Infusion times were 2 minutes for TEA and 5 minutes for adrenergic-blocking agents.

The carotids, over a length of 2 inches or more, were carefully isolated from surrounding structures by blunt dissection. Occlusion was performed by gentle compression of both vessels between moistened fingers for 30 seconds. Care was taken to avoid sensory stimulation through neighboring structures (such as the vagi) or through mechanical stimulation of carotid sinus by stretching the arteries. A minimum of 10 minutes for recovery was allowed between occlusions. Peak change of pressure as a result of occlusion was the statistical criterion employed.

RESULTS

Table 1 shows comparative results in dogs under 3 anesthetic agents. Statistical analysis revealed that neither control pressures nor peak pressor responses to carotid occlusion differed significantly under ether or pentobarbital. On the contrary the difference between control pressures with ether or pentobarbital as compared to those with urethane was highly significant ($t_{91} = 4.08$ and $t_{94} = 5.63$). Similarly the greater

TABLE 1. CONTROL MEAN ARTERIAL PRESSURES AND PRESSOR RESPONSE TO 30 SECONDS' CAROTID OCCLUSION IN DOGS UNDER VARIOUS ANESTHETICS

ANESTHETIC	NO. OF TRIALS IN (NO. OF DOGS)	CAROTID OCCLUSION MEAN, *S*, AND (RANGE)	MEAN. ART. PRESS. MEAN, *S*, AND (RANGE)	CORRELATION OF MEAN AND RESPONSE
		mm.Hg	*mm.Hg.*	
Pentobarbital	49 (28)	23 ± 13 (2 — 55)	118 ± 22 (57 — 174)	0.73
Urethane	47 (39)	35 ± 14 (12 — 68)	143 ± 21 (98 — 182)	0.60
Ether	46 (20)	24 ± 16 (3 — 78)	125 ± 21 (98 — 184)	0.47

carotid response under urethane was highly significant ($t_{91} = 3.03$ and $t_{94} = 3.50$ in the same order). This difference was not greater than that expected under ether or pentobarbital at a control pressure as great as that which existed under urethane. This can be verified by examining figures 1*A*, *B* and *C* which present for each of the anesthetics the individual pressor responses plotted against the pre-existing mean arterial pressure. Figure 2 presents the regression lines of these data. It is seen that projection to $Y = 0$ predicts that carotid occlusion will fail to produce a pressor response at about 60 mm. Hg mean arterial pressure. Thus dogs under urethane had on the average a higher mean arterial pressure than those under ether or pentobarbital[1] but in any case the pressor response to carotid occlusion was some direct function of the pre-existing mean arterial pressure.

The pattern of response to carotid occlusion showed no notable difference under

[1] While these differences in average mean arterial pressure in these series with the 3 anesthetics are significant statistically they can not be interpreted as proof that urethane produces a higher arterial pressure than pentobarbital. This was illustrated in 300 dogs anesthtized with pentobarbital in other experiments in this laboratory without neck surgery. The average mean arterial pressure for the 12 groups of 25 dogs varied from 132 to 144 mm. Hg. The mean of these 12 averages was 139 ± 5.5 mm. Hg. The high standard deviation of the series in the table illustrates that they should not be interpreted for this point.

the 3 anesthetics. The primary rapid rise occurred within 15 seconds as a rule. Thereafter the pressure became level or less commonly continued to rise very slowly. In a small series the duration of occlusion was extended to 60 seconds. The final pressure after 60 seconds was not significantly higher than that after 30 seconds. Release of the arteries resulted in a rapid fall of pressure to the pre-existing control level. In

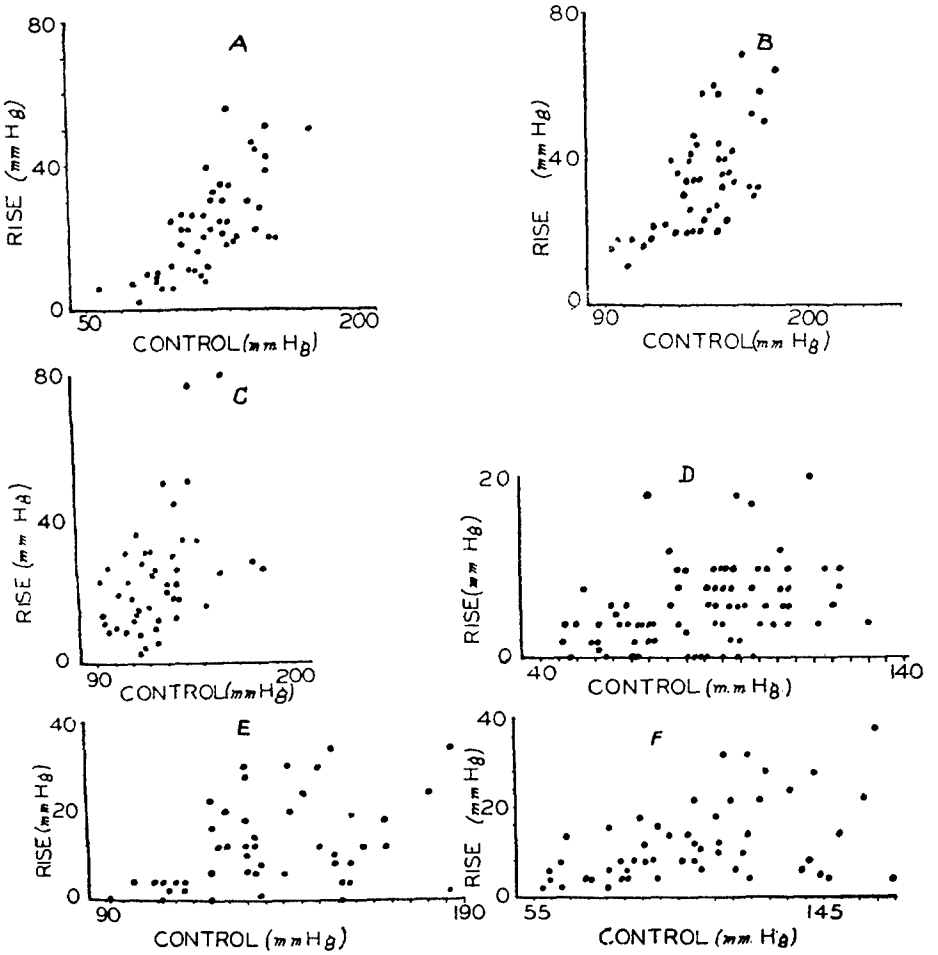

Fig. 1. INDIVIDUAL PEAK MEAN-ARTERIAL-PRESSURE CHANGES due to 30 seconds, carotid occlusion plotted against pre-occlusion mean arterial pressure. *A*) Under pentobarbital, 49 trials in 28 dogs. *B*) Under urethane, 47 trials in 39 dogs. *C*) Under ether, 46 trials in 20 dogs. *D*) After TEA (5–25 mg/kg.) adequate to block peripheral vagal stimulation under pentobarbital. Note difference in scale on ordinate. *E*) After DHO 180 (1–3 mg/kg.), some under pentobarbital some under urethane. *F*) After BBB (2–4 mg/kg.), 20 of 22 under pentobarbital.

about 25 per cent of cases an after-fall to levels below the control was noted. The depth of this fall averaged only 5 per cent of the control pressure and the depression was short-lived. No correlates of the after-fall were found by examining the data. Thus the pattern of response to carotid occlusion in the anesthetized dog was independent of the anesthetics used in this series.

Table 2 shows the effects of tetraethylammonium chloride (TEA) on the response to carotid occlusion under pentobarbital. Due to the fleeting action of the drug occlusion responses were accepted only during such time as right vagal stimulation at two times threshold value failed to produce cardiac slowing. With adequate dosage it

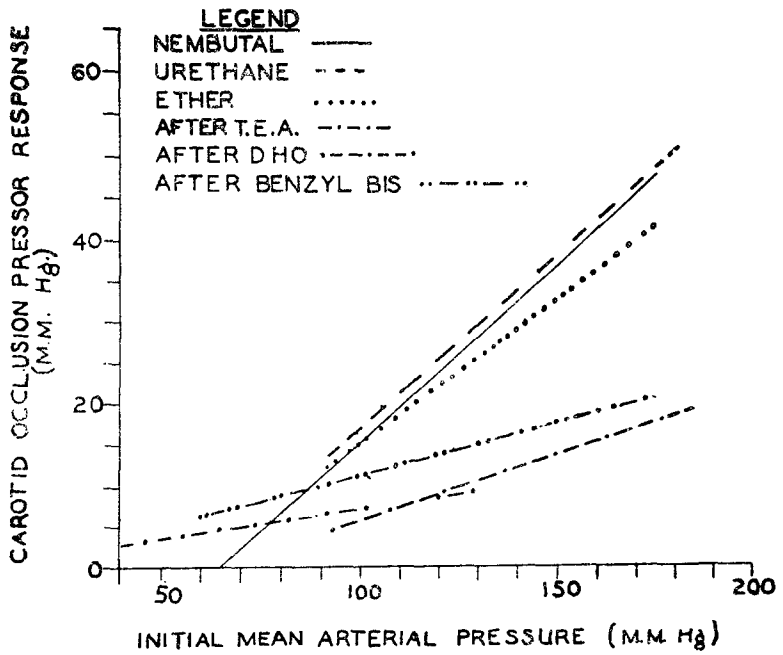

Fig. 2. PLOT OF REGRESSION LINES for data of figure 1 calculated on basis $Y = a - b\,X$, where $b = xy/x^2$ and a is obtained by solution of above formula using mean values of X and Y. X, x are actual mean arterial pressure values and deviations from the average mean arterial pressure, respectively. Y, y are mm. Hg response to 30 seconds' carotid occlusion and deviation from mean response, respectively.

	Value for		Standard deviation of regression line[1]
	a		
			mm. Hg
Nembutal	−28	0.432	± 8.8
Urethane	−25.1	0.417	±11.1
Ether	−20.8	0.358	±14.3
After TEA	0.3	0.067	± 4.1
After DHO	−10.3	0.164	± 9.3
After BBB	− 0.6	0.117	± 8.0

[1] For method see SNEDECOR, G. W. *Statistical Methods* (4th ed.)..Ames: Iowa State College Press, 1946, p. 117.

appeared that 12 or more minutes of blocking action were available after completion of injection of TEA. These data include 35 administrations of TEA (10 at 5 mg., 14 at 10 mg., 7 at 15 mg. and 4 at 20–25 mg.). There was but one administration at 5 mg. and one at 15 mg. which failed to block the vagus. Thus in the dog anesthetized

with pentobarbital, 5 mg/kg. of TEA was an adequate dose to block vagal stimulation and carotid occlusion. Figure 1*D* shows the responses. For comparison table 2 also presents the effect of 5 mg. TEA on 9 animals anesthetized with ether. It is clear that TEA was far less effective at this dosage under ether than it had been under pentobarbital.

The effects of adrenergic blocking drugs on mean arterial pressure and carotid occlusion pressor response are likewise seen in table 2. In the series under DHO 180

TABLE 2. EFFECT OF GANGLIONIC- AND ADRENERGIC-BLOCKING AGENTS ON THE PRESSOR RESPONSE TO CAROTID OCCLUSION IN ANESTHETIZED DOGS

AGENT AND ANESTHETIC	NO. OF TRIALS IN (NO. OF DOGS)	CONTROL		AFTER EFFECTIVE DOSE OF AGENT		
		Mean Art. Press. Av., *S*, and (range)	Carotid Occlusion Peak Rise, Av., *S*, and (range)	No. of Trials in (No. of Admin.)	Mean Art. Press. Av., *S*, and (range)	Carotid Occlusion, Peak Rise, Av, *S*, and (range)
		mm.Hg	*mm.Hg*		*mm.Hg*	*mm.Hg*
TEA 5 mg/kg. ether	18	140 ± 22	35 ± 17	10	127 ± 20	26 ± 23
	(9)	(70–104)	(4–78)	(9)	(92–170)	(7–88)
TEA 5–25 mg/kg. pentobarbital	51	121 ± 26	24 ± 11	78	85 ± 22	6 ± 4
	(18)	(80–160)	(8–47)	(35)	(40–130)	(0–20)
DHO 180[1] 1–3 mg/kg.	25	131 ± 19	34 ± 13	46	136 ± 23	12 ± 10
	(16)	(104–172)	(14–60)	(16)	(92–186)	(0–34)
BBB[2] 2–4 mg/kg.	24	147 ± 18	34 ± 12	52	108 ± 31	12 ± 9
	(22)	(110–176)	(16–58)	(22)	(58–174)	(2–38)

[1] Ten under pentobarbital, 6 under urethane. [2] Two under urethane, 20 under pentobarbital.

TABLE 3. DATA OF AVERAGE REGRESSION LINE TREATED TO ELIMINATE EFFECTS OF INITIAL MEAN ARTERIAL PRESSURE

MEAN ARTERIAL PRESSURE (*a*)	CAROTID OCCLUSION RESPONSE (*b*)	CAROTID OCCLUSION AS % MEAN ART. PRESS $b/a \times 100$	CAROTID OCCLUSION AS % of (MEAN ART. PRESS.−60) $b/a - 60 \times 100$
	mm.Hg		
60	0	0	0
80	8	10	40
100	16	16	40
120	24	20	40
140	32	23	40
175	46	26	40

(fig. 1*E*) the dose used varied between 1.0 and 3.0 mg/kg. but no relation between dose and degree of blockage of carotid response was evident. Duration of blockage seemed to increase at higher doses. These doses produced an epinephrine reversal classed as fair[2] 6 times, good 6 times, and complete 2 times in the 16 dogs. From figure 2 it can be seen that the regression line illustrated good blockage of the carotid pressor

[2] Epinephrine reversal was tested by i.v. injection of 5 μg/kg. Response was classed as 'poor' if pressor component was more than 50 mm. Hg and after-fall was markedly accentuated, 'fair' if pressor was 20 to 50 mm. Hg and after-fall strong, 'good' if pressor response was less than 20 mm. Hg and 'complete' if only a depressor response resulted.

response. The blockage was incomplete in only 4 of 16 dogs. These 4 animals had dosages of 2 or 3 mg/kg.

Similarly with 2 to 4 mg/kg. of BBB suppression of carotid-occlusion-pressor response was incomplete in 6 of 22 animals (fig. 1*F*, 2). Epinephrine reversal was 'good' to 'complete' with this drug in 17 of 19 dogs.

In summary peripheral and central adrenergic-blocking and ganglionic-blocking agents had the power to reduce sharply the response to bilateral carotid occlusion in the anesthetized dog and to alter the direct relation between mean arterial pressure and carotid pressor response.

DISCUSSION

W. T. Porter demonstrated in 1907–08 (9) for several stimuli that reflex blood pressure response was quantitatively related to the level of blood pressure which existed when the reflex was evoked. Following his example it became common to report blood pressure changes in percentage of pre-existing pressure rather than in absolute values. This was done in order to reduce variability between observations by reducing to a common denominator. The practice is valid so long as the reflex used has been shown to display a direct relationship; the reporting of percentages will tend to conceal the relationship so that it becomes necessary to prove the proportionality for each reflex.

The fact that the regression lines for all 3 anesthetics project back to the zero response line near 60 mm. Hg mean arterial pressure correlates well with existing knowledge of the function of the carotid sinus. Many workers (1) have shown that distention of the isolated carotid sinus caused no reflex blood pressure fall unless the distending pressure exceeded 45 to 60 mm. Hg. Bronk and Stella (10) showed that few afferent impulses traversed Hering's nerve unless the distending pressure exceeded 40 to 50 mm. Hg. Thus in the intact animal, at a low level of arterial pressure, withdrawal of reflexogeneous impulses due to occlusion of the carotid could not occur, and reflex effects of bilateral carotid occlusion should rarely be seen.

The existence of this unresponsive zone (0–60 mm. Hg within which change of mean arterial pressure will not alter the carotid occlusion response) will prevent unification by direct reduction to percentage of results obtained at different pressures. This is clearly seen if the data from the regression line are reduced to percentage as in table 3. This very pleasing result is not unexpected since the process of drawing a regression line tends to minimize all variations extraneous to the correlate. If individual results are to be treated by this means these variables must be evaluated. The standard deviations of the regression lines seen in the caption of figure 2 offer an estimate of this residual variability. That it is still sizeable does not negate the value of the reduction gained. It is concluded that comparisons of data on carotid-occlusion-pressor responses obtained at different levels of mean arterial pressure can be facilitated by *a*) use of the regression lines or *b*) reporting as (mm. Hg rise due to occlusion $\times$ 100)/(mean arterial pressure (mm. Hg) $-$ 60).

Examination of data of individual dogs under adrenergic-blocking drugs showed that instances occurred sporadically under BBB or DHO 180 in which carotid pressor response was only partially suppressed though epinephrine reversal was complete. The reverse has been seen only under DHO 180. This suggests that the process of

suppressing carotid pressor response under DHO 180 is separate from the process of reversing epinephrine. This correlates with the postulation of Bluntschli (11) that DHO 180 attacks the sympathetic pathway centrally.

SUMMARY

In anesthetized dogs it was found that the pressor response to bilateral occlusion (30 seconds) of the carotid arteries was directly related to the pre-existing mean arterial pressure, at least within the limits of 70 and 160 mm. Hg. The relationship between pressure and response was not different in dogs under pentobarbital or urethane intravenously administered nor under ether by inhalation. Tetraethylammonium chloride (5–15 mg/kg.), dihydroergocornine (DHO 180) (1–3 mg/kg.) or benzyl bisβchloroethyl amine (2–4 mg/kg.) was shown to suppress the pressor response to carotid occlusion and to alter the relationship of response to mean arterial pressure.

Regression lines interpreting the carotid occlusion pressor response as a function of mean arterial pressure have been presented for each of the aforementioned conditions. These lines approach o response at 60 mm. Hg pressure. Little response to carotid occlusion is to be expected from the anesthetized dog when mean arterial pressure is below 80 mm. Hg. It was concluded that minimum variability will be obtained if carotid-occlusion-pressor responses in future work are reported as change of MAP due to occlusion × 100/initial MAP − 60 in percentage.

The authors desire to express their gratitude to Dr. K. E. Penrod for his help with statistical methods utilized in this work.

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SECTION III

HYPOXIC ALTERATIONS IN BLOOD PRESSURE AND PULMONARY VENTILATION IN THE ANESTHETIZED DOG: RELATION OF ADRENERGIC BLOCKADE

Barbara Brown, J. W. Stutzman and George L. Maison

The results presented below were obtained in the process of an investigation of the effects of agents influencing the autonomic nervous system on the reactions of animals to the stress of oxygen want. Many reports bear witness to the concept that hypoxia causes a pressor response which is reversed by adrenergic blockade. The pressor response has been found to depend on the integrity of the sympathetic division of the autonomic nervous system in both its vascular tonic and its epinephrine discharging aspects. It is clear from the literature that there is great variability of the magnitude of the pressor response but neither quantitative measures of the variability nor evidence of the source of the variability has been found. No validation of methods of reporting the change of blood pressure (as mm Hg change of mean arterial pressure, MAP, or as % change of MAP from pre-existing pressure) has appeared. The influence of different anesthetics on the response has not been outlined.

The same statements of paucity of quantitative information hold for pulmonary ventilatory alterations due to hypoxia in laboratory animals. Most of the reports in the literature are complicated by variable rates of change of stress as in the rebreathing techniques with carbon dioxide reabsorption in a small reservoir. Others present a time of stress so short as to prevent any real adaptation of the animal to the stated conditions. The only previous systematic attack on the autonomic nervous system by means of drugs using response to hypoxia as criterion was limited to a stress period of one minute. (1)

The experiments which follow were designed to delineate alterations due to low oxygen exposure uniform over 5 minutes; to seek differences in alterations shown by animals anesthetized with two common laboratory anesthetics; to seek differences in alterations following adrenergic blockade due to agents of β chloroethylamine[1], of imidazoline[2] and of ergot[3] chemical structure.

METHOD

Dogs were anesthetized with urethane 1.4 to 2.2 Gm/Kg or pentobarbital sodium ca 30 mg/Kg intravenously.

Arterial blood pressure was recorded from the femoral artery by means of a mercury manometer, and injections were made via a cannula in the ipsilateral jugular vein. The trachea was cannulated and connected to a polarized flutter valve system. Respiration was recorded qualitatively by means of a rubber tambour attached to a side arm of the tracheal cannula. Recordings were made on Teledeltos paper moving at a rate of approximately 10 mm/minute.

1/ n-n-dibenzyl β chloroethylamine HCl, dibenamine (Dbn)

Benzyl bis β chloroethylamine HCl (BBB)

n-n-bis-(o-methylphenoxyethyl)-β chloroethylamine HCl (186)

1-naphthyl-methyl ethyl β chloroethylamine HCl (121)

and as impotent congener control drugs

n-n-dibenzyl hydroxyethylamine HCl (NOH)

n-chloroethyl isoindole (184)

2/ Benzyl imidazoline HCl - Priscoline (Pris.).
2(N paratolyl Nmetahydroxyphenyl-aminoethyl) imidazoline HCl (7337).

3/ Dihydroergocornine (DHO 180).

Hypoxia was induced by permitting the animal to breathe a nitrogen-oxygen mixture from a reservoir consisting of a large rubber bag of 1500 liter capacity. Its contents were analyzed repeatedly during the day by means of a Pauling oxygen analyzer and/or modified Haldane gas analyzer. The oxygen ranged from 5 to 11%. Hypoxial trials were of 5 minutes' duration, with a period of at least 15 minutes between trials.

When carbon dioxide was added to the mixture, a spirometer of 300 liter capacity was used as a reservoir. The carbon dioxide content ranged from 2.6 to 5.0%.

Transfers from room air to gas mixture and vice versa were made instantaneously. Orifices of uniform size provided access to inspired atmosphere and minimized differences in resistance to breathing.

In one series of experiments there was added to the above procedures the registration of minute ventilation responses in cu. ft. per minute by means of a Wet Gas Test Meter attached to the output valve. The hypoxial trials were preceded and followed by 5 control readings of ventilation while breathing room air.

Epinephrine 5 to 10 mcg/Kg was injected as an indicator of the sensitivity of the adrenergic end organs before and after the administration of adrenergic blocking drug.

Adrenergic blocking agents were prepared by solution in equal parts of propylene glycol and alcohol and dilution with saline. The agent was infused over a period of 5 to 20 minutes by vein. When necessary the dogs were resuscitated by means of a positive pressure pump or by manual compression of the chest.

The criterion chosen for blood pressure response was peak rise or fall in millimeters of mercury of mean arterial blood pressure; for ventilation, was the average per minute output in liters per square meter over the period. Surface area was calculated by the formula of Meeh (2). Each animal served as his own control.

TABLE 1

The Effect of Anesthetics on Response of Mean Arterial Pressure of Dogs to 5-Minute Exposure to Hypoxia

Anesthesia	No. of Trials in (dogs)	Control Average and (Range) mm Hg	Hypoxia Response MAP Avg. and (Range) mm Hg	% Oxygen *
Urethane	85 (58)	134.5 (77 -180)	+15.2 (-6 - +54)	8.0 ± 0.7% (6.0 - 8.8)
Pentobarbital	30 (22)	119.9 (60 -192)	+16.9 (-14 - +46)	7.3 ± 0.5% (6.6 - 8.3)
Total	115 (80)	130.7 ± 24.4 (60 - 192)	+15.7 ± 11.2 (-14 - +54)	(6.0 - 8.8)

* Twentyfour experiments on 13 animals at 6 - 6.9% O_2; 33 experiments on 22 animals at 7 - 7.9% and 58 experiments on 45 animals at 8 - 8.8%.

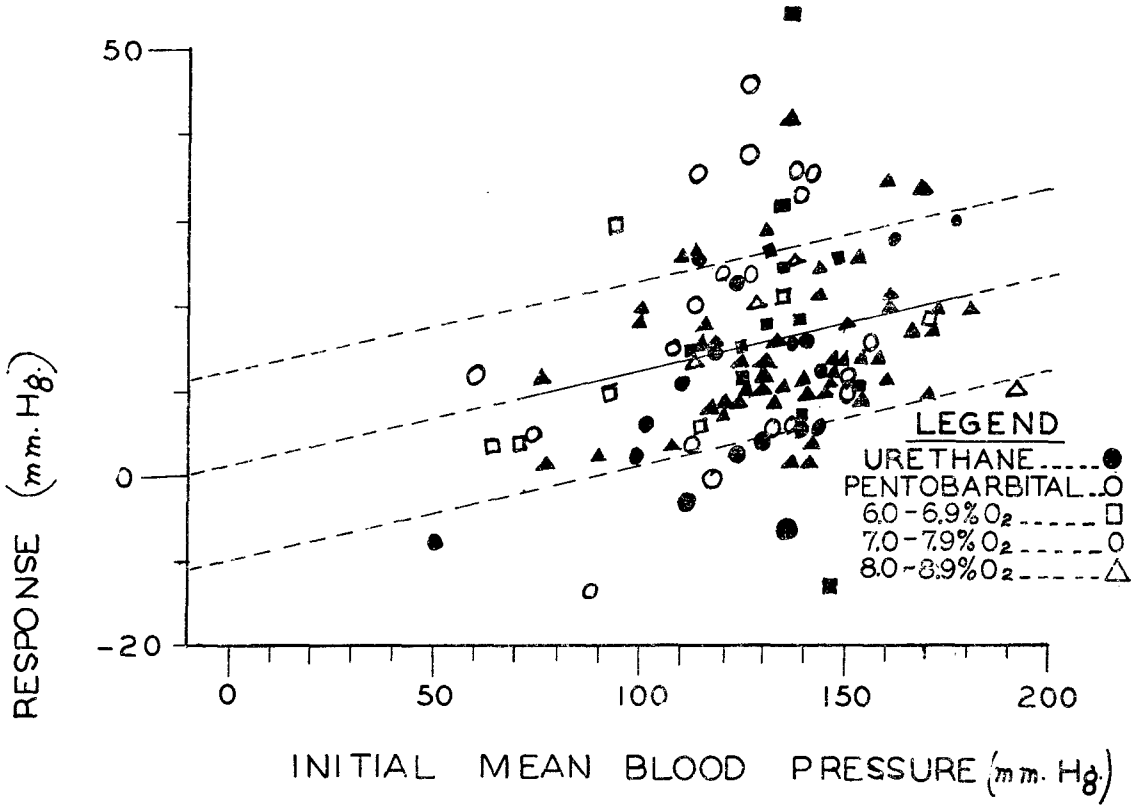

Figure 1. Plot of peak alteration of mean arterial pressure during 5 minutes' exposure of anesthetized dogs to 6 to 9% oxygen against initial mean arterial pressure. Center line is regression line calculated according to formula $Y = a + b X$ and $b = \frac{xy}{x^2}$. The line is projected beyond existing data (dashes). Outer lines represent the standard deviation of this regression calculated according to Snedecor, "Statistical Methods" (4th ed.) 1946, p. 117. Inspection shows that (1) animals anesthetized with pentobarbital had lower MAP and (2) that little correlation between percentage oxygen and magnitude of response occurred over this narrow range.

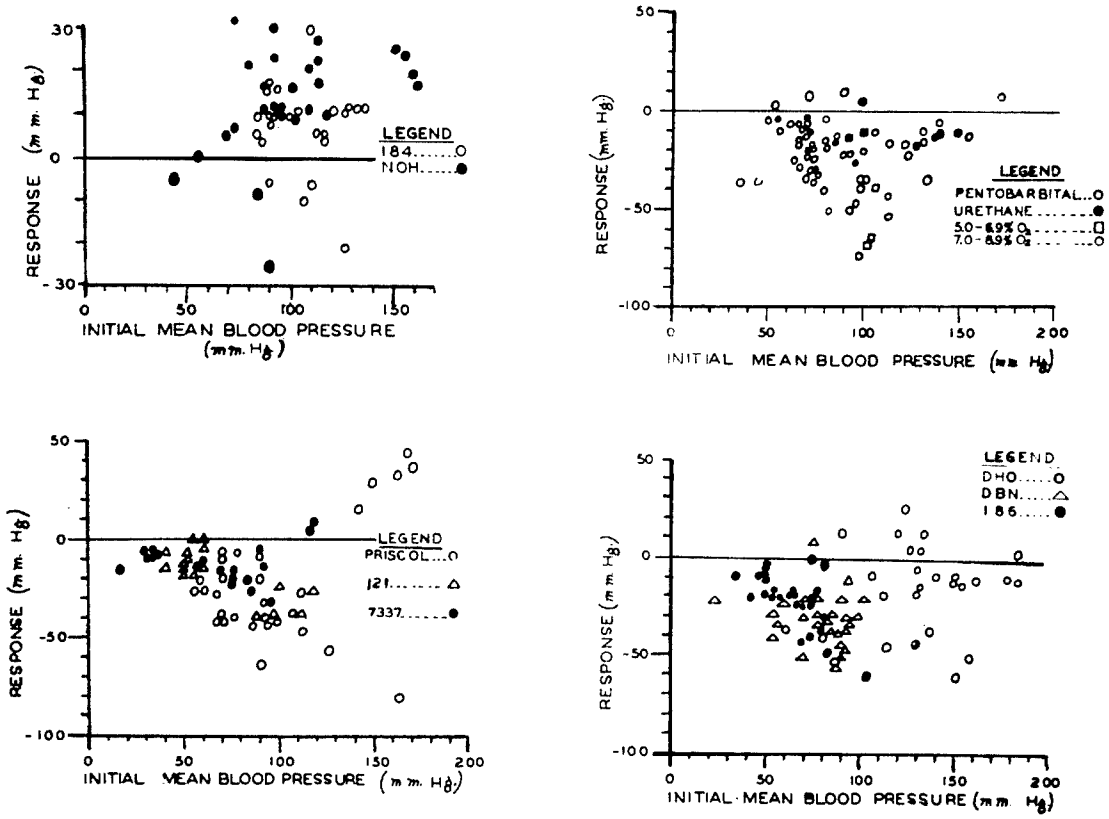

Figure 2. Same as for Figure 1 but after animals had been treated with certain drugs.

Top left: After impotent congeners of β chlorethylamine blocking drugs.

Top Right: After benzyl bis β chloroethyl amine. See "Methods" section for other symbols.

TABLE 2

Incidence of Various Patterns of Mean Arterial Blood Pressure Response to Hypoxia* in Anesthetized Dogs

Condition	No. of Trials	Depressor with or without a preceding short-lived small rise	Pressor short-lived and depressor in < 5 minutes	Pressor increasing throughout 5 minutes or forming plateau at high level; never depressor	No change
Control	115	5.25%	10.4%	81.8%	2.6%
Impotent congeners of adrenergic blocking agents	47	0	19.0	76.5	5.2
Adrenergic blocking agent	152	92	3.3	1.3	3.3

* O_2 level 6.0 - 8.9%

TABLE 3

Ventilation Volume of Anesthetized Dogs Before, During and After Hypoxia of 5 Minutes' Duration Induced by Exposure to Oxygen-Poor Air Mixtures

Anesthetic	Drug	Inspired Mixture Avg. and S	CONTROL No. of Trials (in dogs)	CONTROL Liters/ sq. m./ min.	HYPOXIA No. of Trials (in dogs)	HYPOXIA Liters/ sq. m./ min.	AFTER HYPOXIA No. of Trials (in dogs)	AFTER HYPOXIA Liters/ sq. m./ min.	% rise of Ventilation on shift to hypoxia
Urethane	0	O_2% 8.17 ± 0.46 N_2	44 (18)	8.7 ± 4.5	31 (17)	14.5 ± 5.6	30 (17)	9.3	66
Pentobarbital	0	O_2% 7.11 ± 0.41 N_2	16 (10)	3.5 ± 1.8	15 (10)	7.8 ± 5.5	15 (10)	2.9	126
Urethane	0	O_2% 8.18 ± 0.54 N_2	11 (11)	7.0 ± 4.0	10 (10)	12.3 ± 3.8	10 (10)	7.9 ± 2.9	75
Urethane	0	02% 8.18 ± 0.54 CO_2 4.0 N_2	11 (11)	6.9 ± 2.3	11 (11)	11.9 ± 4.1	10 (10)	9.8 ± 4.6	72
Urethane	0	O_2 8.1 ± 0.51 N_2	13 (11)	8.8 ± 4.3	13 (11)	14.4 ± 5.6 *	13 (11)	9.5 ± 3.5	63.5
Urethane	BBB	O_2 8.1 ± 0.51 N_2	17 (11)	9.9 ± 2.7	17 (11)	11.5 ± ** 4.7	10 (6)	10,0	16.2

* Maximum ventilation rate for any whole minute 17.8 ± 7.8 L./sq. m./min.
** " " " " " " " 13.6 ± 5.3 L./sq/ m./min.

RESULTS

Responses of anesthetized Dogs to low oxygen mixtures: Table 1 presents the average results of control exposures to hypoxia in animals anesthetized with the two anesthetics. In no case did the animal fail to maintain spontaneous respiration throughout the 5-minute trial of hypoxia (6.0 to 8.9% oxygen range). It will be noted that the average rise of MAP was similar with both agents despite the different level of control MAP. The individual trials (115 in 80 dogs) are plotted against pre-existing MAP in Figure 1. Regression line plotted shows that average rise of MAP is a direct function of the pre-existing pressure ($r = +0.237$)[4]. The data of the regression line at all points showed peak response to be 11.6 to 14% of the control MAP. Therefor it is proper to report peak blood pressure responses to hypoxia as percentage of control pressure. The low correlation coefficient emphasizes, however, that only a small part of the variability of blood pressure response to hypoxia will be eliminated by this means. Close examination of the data suggested that neither the kind of anesthesia nor the percentage of oxygen used within this narrow range significantly altered the response.

The pattern of blood pressure change induced by the hypoxia included a pressor alteration in 95.6% of the trials. In 92% the rise was the predominant change. In 82% the blood pressure was above the control level throughout the 5-minute exposure and during recovery on room air. Only 2% of the trials showed a pure depressor reaction. 2.4% showed no change. Table 2 illustrates these facts.

Rates of ventilation were measured in a smaller series. Table 3 shows a marked discrepancy in rate of ventilation of room air by animals anesthetized with pentobarbital as compared to those anesthetized with urethane. The same discrepancy is noted in the ventilation response to 5 minutes of hypoxia. In

[4] With n = 116 this value has a p below 0.01

spite of this difference, percentagewise there was a larger increase due to hypoxia in the dogs anesthetized with pentobarbital than in those with urethane.

The wide variability of ventilation rates from one dog to another in these trials is noteworthy. In a single dog in which 10 such 5-minute exposures to hypoxia were done consecutively the average ventilation on room air was 6.2 ± 0.82 liters per sq. m. per minute. The small standard deviation suggested that factors differing between dogs were responsible for the large standard deviation of the total series. However, examination of the different room air ventilation rates in a series of 16 dogs exposed twice to such measurement within one hour showed that the rate deviation between trials was more than 33% of the average rate in 5 of the dogs. It is still possible that inaccuracies in estimation of surface area (based purely on body weight) account for a part of the variability between dogs.

Pattern of ventilatory response to 5 minutes' hypoxia (6 to 8.9% oxygen in nitrogen) included a rise in all cases. In animals under urethane half of 33 trials showed ventilation increasing throughout the 5-minute period. The remainder showed peak ventilation in the second to fourth minute of exposure. Only 3 trials of the 33 presented a failure to maintain the high ventilation rate with a fifth minute ventilation back down to the room air control rate. The pattern of ventilation during hypoxia in animals anesthetized with pentobarbital was not appreciably different from the above despite the marked quantitative difference in volumes.

A further series of animals was undertaken to determine whether the presence of CO_2 in the hypoxic mixture would improve the ventilatory response. Table 3 also shows these results. It is seen that the rise of ventilation due to hypoxia was not augmented by addition of 2.6 to 5% CO_2 (average 4%). Suggestive is the fact that on return to room air after hypercapnic hypoxia ventilation remained

TABLE 4

Effect of Adrenergic Blocking Drugs and Impotent Congeners on Mean Arterial Blood Pressure and Respiratory Responses to Hypoxia of Anesthetized Dogs

	BEFORE DRUG			AFTER DRUG				
Drug* and (Dose) mg/Kg	#Trials in (#Dogs)	Control MAP mm Hg	Change due to Hypoxia mm Hg	#Trials in (#Dogs)	MAP before Hypoxia mm Hg	Change due to Hypoxia mm Hg	Incidence of Respiratory arrest	Avg % O_2 used
NOH (6 to 12)	15 (8)	127	+10.5	28 (8)	100	+12.7	2 of 28	8.1
184 (18)	12 (6)	125	+12.4	24 (6)	105.3	+ 7.6	0 of 24	8.4
BBB (1 to 9)	29 (23)	144	+16.8	47 (23)	90.7	-23.6	29 of 47	8.2
BBB (3)	7 (7)	152.9	+22	10 (7)	86.4	-31.8	10 of 10	O_2 8.1 CO_2 4.0
121 (2 to 3)	12 (6)	133.6	+11.8	17 (6)	67.1	-16.2	11 of 17	9.7
Di B (12 to 24)	12 (6)	128.9	+17.3	28 (6)	78.9	-30.1	13 of 28	7.1
186 (6 to 12)	19 (14)	142.8	+14.2	28 (14)	67.7	-18.9	20 of 28	8.3
7337 (2 to 5)	14 (7)	125.1	+15.3	19 (7)	66.6	-10.8	9 of 19	8.1
Priscol (10 to 20)	15 (6)	135.3	+24.8	19 (6)	90.5	-38.4	16 of 19	6.4
DHO 180 (10 to 20)	8 (8)	145.5	+23.5	26 (8)	131.7	-14.0	3 of 26	8.3

*The dosages reported are cumulative, yet the first dose was always an effective dose from viewpoint of epinephrine reversal except in the cases of NOH and 184. Epinephrine reversal was classed as good or complete (see definition elsewhere in this paper) in 107 out of 117 trials in the 88 dogs given potent drugs. Of the remaining 10 trials 8 were classed as fair, one as poor and one as identical with control trial. By contrast in the 14 dogs which received impotent congeners 17 trials showed one classed as good reversal 7 as fair 1 as poor and 8 as identical with control trials.

high longer than after hypoxia alone.

The interrelation of blood pressure and ventilatory responses was analyzed in 31 hypoxic trials in 17 dogs anesthetized with urethane. The correlation coefficient between percentage change of MAP and percentage change of ventilation proved to be +0.37 which approaches, but does not exceed, the 5% level of significance. Thus in general a good rise of blood pressure in response to hypoxia is attended by a good rise of ventilation but many other variables interfere with predictability.

The influence of depth of anesthesia on the variability shown in these blood pressure and ventilatory responses to hypoxia is difficult to estimate. One effort in this direction was to plot the amount of urethane per kilogram of body weight against the blood pressure and ventilatory responses. The result was a scatter so broad as not to justify reproduction. It seems probable that blood level of anesthetic and not total dose would be required to approach the question.

Responses to hypoxia after treatment with adrenergic blocking drugs: Table 4 presents the results of 5-minute exposure to hypoxic mixtures of a series of dogs before and after treatment with adrenergic blocking drugs. The data show that adequate dosage of these agents robbed the animals of the ability to maintain respiration for 5 minutes under conditions of hypoxia which were easily survived by the same dog before the drug. This phenomenon is not the subject of the present analysis. It will be discussed in detail elsewhere.

Blood pressure responses seen in Table 4 show that: (1) the "impotent" congeners (184, NOH) of adrenergic blocking drugs even in large dosage did not reverse the hypoxic pressor response though they did lower the control MAP to a significant extent.

(2) Six of the 7 potent adrenergic blocking drugs tested caused significant lowering of MAP in dogs anesthetized with urethane. Inadequate results suggested that same occurred in dogs under pentobarbital anesthesia. DHO 180 was the exception.

(3) Each of the 7 agents reversed the pressor action of hypoxia. In general the extent of the depressor reaction to hypoxia after adrenergic blockade was greater in animals anesthetized with urethane. This was most strikingly seen with Priscol which reversed the reaction in only half of the trials with pentobarbital anesthesia. The degree of depressor reaction after the drugs correlated poorly with the averaged oxygen tension in the inspired air.

The average extent to which the individual drugs lowered the MAP correlated poorly with the average degree of depressor response evoked by hypoxia under the drugs. Yet separate plots for each drug of the blood pressure alteration due to hypoxia against the pre-hypoxial blood pressure in each trial suggested that the depressor response was closely correlated with the pre-existing blood pressure. See Figure 2 (composite 4 graphs). The pattern of blood pressure response after the impotent congeners and the potent adrenergic blocking drugs was presented in Table 2. The alteration was striking.

The effects of adrenergic blocking agents on the ventilation volume was adequately investigated only for BBB. Data are presented in Table 3. The differences between analogous means before and after the drug were not significant by T test yet the trend toward reduction of the ventilatory response to hypoxia by adrenergic blocking drugs appeared provocative. The low percentage change of ventilation due to hypoxia in presence of adrenergic blocking drugs was significant.[5/] This reflected the fact that 5 animals out of 11 showed an actual decrease of ventilation on exposure to hypoxia after treatment with the drug.

The dosage of BBB was 1 mg. or more per kilogram of body weight but was not held constant in these trials on a weight basis. Rather it was judged by the degree of epinephrine reversal. Only one reversal classed as poorer than fair was accepted in this series.[6/]

5/ Other experiments not reported here showed that tetraethylammonium bromide in dosage adequate to block both carotid occlusion pressor response and the response to peripheral vagal stimulation did not significantly reduce the rise of ventilation on exposure to 8% O_2 in N_2 (8 dogs anesthetized with pentobarbital).

6/ Epinephrine reversal was classed as complete if response was entirely depressor, good if pressor component were less than 20 mm Hg with marked depressor component, fair if 20 to 50 mm Hg pressor effect with marked depressor component occurred and poor if pressor response was greater than 50 mm Hg.

DISCUSSION

These results showed that adrenergic blockade in the anesthetized dog prevented the maintenance of respiration of oxygen-poor air at concentrations and for times easily withstood in the absence of blockade. This is identical with results previously reported in the rabbit with and without anesthesia (3). This phenomenon will receive more extensive treatment along with other experiments which attempted elucidation of mechanism of the respiratory failure.

The method of abrupt exposure to a fixed oxygen-poor mixture for a set time has the advantage of permitting development of a steady state if the animal can adapt. It has the disadvantage that the adaptation is demanded immediately. There is no gradual transition. It is analogous to a flyer's experience in being abruptly deprived of his oxygen supply while in level flight at a high altitude and not analogous to a gradual ascent in low-pressure chamber or aircraft. Other data to be published showed that dogs with adrenergic blockade under identical conditions can adapt if presented with a gradual transition to oxygen-poor conditions.

The classification of patterns of blood pressure response to hypoxia involved certain assumptions. It was assumed that a rise of MAP in the face of reduced partial pressure of inspired oxygen was the normal response and an evidence of a purposeful reflex in substituting more rapid traverse of blood through vital organs for a larger arteriovenous oxygen difference. It was assumed, second, that failure to maintain MAP was evidence of failure to adapt. Such a division left a small group of cases uncovered by the assumptions. These showed no change of MAP during 5 minutes of hypoxia. This could represent (1) a mechanical failure - leakage and no hypoxia presented, (2) inability to respond - a poor state, or (3) a circulatory adjustment so good that the hypoxia presented was a stress inadequate to provoke a change. While the latter interpretation seems preferable, evidence for it is wanting. Fortunately the incidence proved to be negligible. The sharp reversal of pattern after the blocking drugs is not new though quantitative analysis of it has not been found in the literature.

The magnitude of the rise in MAP (16 mm Hg) evoked by hypoxia (8% O_2) in these anesthetized dogs compared well with the 30 mm Hg average reported by Surtshin et al. (4) for dogs anesthetized with pentobarbital and exposed to pure nitrogen. The magnitude of the fall in MAP evoked by hypoxia after the blocking agents appeared to be greater than the magnitude of the rise evoked before the drug. For all of the drugs except DHO-180 the magnitude of the fall appeared to be roughly proportional to the state of the blood pressure before the hypoxia was induced. This is another way of saying that if the drug depressed the blood pressure to a low level little fall could occur.

Speculation is evoked by comparing the average MAP responses to epinephrine (average 10 mcg/Kg.) before (66 mm rise in 118 trials) and after blockade (33 mm fall). These results were taken irrespective of the blocking agent used. Such data should bear on the unsettled question as to the contribution made by reflex release of endogenous epinephrine to the rise of blood pressure evoked during hypoxia. The trend shown by these data suggested that other mechanisms besides endogenous epinephrine release must be operating.

It is generally recognized that barbituric acid derivatives such as pentobarbital are depressants of external respiration (Wang and Nims, 5) and of blood oxygen content (Beecher (6), Penrod and Hegnauer (7). The latter phenomenon has been shown to depend on two processes - blood dilution and poor oxygenation due to inadequate ventilation. The poor response of ventilation to hypoxia in dogs anesthetized with pentobarbital in this series reflected these changes in all probability. The comparative results for pentobarbital and urethane confirm the observations on cats by Wang and Nims (5). The possible implication of these data for aerial transportation of patients heavily sedated with barbiturates is thought provoking. The great magnitude of ventilation per square meter of surface area in the dogs anesthetized with urethane raised questions (1) as to whether these were normal

figures and (2) as to whether the surface area determined purely on weight in a mongrel dog has accuracy adequate for derivation of such data.

The data suggest, but due to variability do not prove, that adrenergic blockade increased resting ventilation and decreased ventilatory increase on exposure to hypoxia. It must be realized, however, that the result criterion for each dog was the average per minute ventilation for five or less minutes, during which the animal breathed the hypoxic mixture. In those cases where arrest of ventilation occurred late there was often a good response at first followed by depression. For this reason in Table 3 maximal single ventilation responses to hypoxia before and after adrenergic blockade are supplied. These figures do not eliminate the trend toward poorer ventilation on hypoxia after blockade. It would be desirable to correlate the oxygen carriage with the ventilation during hypoxia of this type in animals anesthetized with urethane and in others with pentobarbital.

No clear alteration was effected by adding 4% CO_2 to the hypoxic mixture as shown in Table 3. Suggestive is the trend to prolongation of the hyperpnea by CO_2. This is compatible with the idea that such animals would accumulate CO_2 which would reflexly induce hyperpnea after return to room air. In other regards these results are not "expected". It is generally assumed that animals exposed to hypoxia without CO_2 should blow off CO_2 during the hyperventilation consequent on the hypoxic stimulus to the carotid body. It is conceived that the respiratory center is somewhat depressed by the low pO_2 and that the reduction of pCO_2 would further the depression. It would be expected that addition of CO_2 to the inspired mixture would improve the ventilation. Blood gas studies would be required to elucidate the mechanism.

The final column of this table giving percentage change of ventilation on exposure to hypoxia may be confusing unless it is realized that these percentages have only suggestive force since (1) when normal ventilation was high a rise of percentage was obviously limited and vice versa and (2) these were obtained by calculations from data of ventilation volumes in liters/sq.m./minute which have large standard deviations. The percentage change on hypoxia after adrenergic blockade was, however, a striking one.

SUMMARY

1. The response to hypoxia (ca.8% O_2 in N_2) of 5 minutes duration of dogs anesthetized with urethane by vein has been contrasted with that of dogs anesthetized with pentobarbital by vein. The characteristic rise of blood pressure was identical in both. The ventilatory alteration was greater in absolute terms but smaller in terms of proportion for the former than for the latter.

2. It has been shown that there was a direct relation between existing blood pressure and the pressor response which occurred on hypoxia in these animals. It is therefore proper to report pressor responses to hypoxia in percentage of initial mean arterial pressure. The data showed that only a small part of the variability of response was due to this factor.

3. The rates of ventilation of anesthetized dogs in liters per square meter of body surface per minute were found strikingly low in animals anesthetized with pentobarbital and strikingly high in those anesthetized with urethane.

4. The quantitative responses of anesthetized dogs after adrenergic blockade have been determined using a variety of blocking agents. It was found that the change of mean arterial pressure was depressor for all agents. After blockade all the agents reversed the pressor response to hypoxia. The response of ventilation was determined after benzyl bisβchloroethyl amine only. It was seen that the room air-ventilation was higher and the change due to hypoxia lower in this series. The addition of 4% CO_2 to the hypoxic mixture did not alter the reactions significantly.

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SECTION IV

RESPIRATORY ARREST IN RABBITS AND ANESTHETIZED DOGS EXPOSED TO HYPOXIA AFTER ADRENERGIC BLOCKADE

George L. Maison, Eben Dustin and J. W. Stutzman

In a previous report (1) it was shown that unanesthetized rabbits pretreated with dibenamine and exposed to hypoxia by an abrupt transition from 20% to 5% oxygen in the inspired air could not maintain external respiration for a five-minute period. Control exposures to hypoxia under identical conditions before dibenamine did not produce respiratory arrest.

The experiments described below detail extension of the observation to other adrenergic blocking drugs and the production of the same phenomenon in the anesthetized dog. Efforts to elucidate the mechanism are described. The resulting data have negative rather than positive implications. It has not been possible to prevent the arrest by carbon dioxide nor pilocarpine. Hypotension has been an almost constant concomitant of respiratory arrest, but it has not been possible to indict conclusively the hypotension as causative of the arrest. Raising the mean arterial pressure (MAP) by aortic occlusion or Pitressin reduced the incidence of arrest. It was shown that the abruptness of transition to hypoxic conditions was essential for production of arrest. Presented with a gradual ascent in the altitude chamber both rabbits and dogs unanesthetized with adrenergic blockade were able to adapt to the hypoxia and respiratory arrest did not occur.

Procedures: Methods utilized in experiments on rabbits and anesthetized dogs have been detailed in previous publications (1, 2, 3). Aortic occlusion was produced by clamp compression just below the diaphragm and the inferior venacava was clamped about 30 seconds later in each case.

b) Low pressure chamber experiments were performed in a standard "six man chamber with lock". Careful attention was paid to ventilation of the chamber and to maintenance of uniform rates of ascent. The pattern of flight was slow to 5000 feet pressure altitude (f.p.a.) (about 2000 feet per minute) then rapid ascent (about 15,000 feet per minute) to 25,000 f.p.a. which was held for five minutes. After rapid descent a ten-minute rest at ground level was allowed. This was followed by similar ascent and exposure at 30,000 f.p.a.

Results: Tables I and 2 illustrate the incidence of arrest of respiration resulting from exposure to hypoxia for five minutes in animals after adrenergic blockade with the various agents.[1/] They illustrate also the absence of this phenomenon after administration of impotent congeners of the betachloroethylamine derivatives. Each of the animals involved had been subjected to one or two trials of hypoxia before drug administration. No respiratory arrest occurred in control exposures in the dogs and only rare instances in the rabbits. Thus this inability to adapt to hypoxic stress was neither species nor agent specific.

The pattern of respiratory arrest in these dogs was at variance with that previously described (1) for rabbits. The resting ventilation volume per minute was greater in dogs after than before adrenergic blockade (see 2). The relative and absolute increase in ventilation volume evoked by hypoxia was less after adrenergic blockade. As the hypoxia was continued there was gradual reduction of the inspiratory and expiratory volume with a gradual but not marked decrease in respiratory rate to apnea. Late in the hypoxic period bradycardia was observed. During the apneic period in some dogs there were occasional feeble gasps but no effectual respirations. Restoration to room air without artificial

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1/n-n dibenzyl β chloroethylamine HCl (dibenamine) DiB); Benzyl bis β chloroethylamine HCl, (BBB); n-n-bis-(o-methyl phenoxyethyl)-β chloroethylamine HCl (186); 1-naphthyl-methyl ethyl β chloroethylamine HCl (121) and as impotent congener control drugs, n-n dibenzyl β hydroxyethylamine HCl (NOH), n-chloroethyl-isoindole (184), Benzyl imidazoline HCl-(Priscoline)-(Pris) and 2 (n paratolyl n meta-hydroxy phenyl - aminomethyl) imidazoline HCl (7337), and Dihydroergocornine (DHO 180).

TABLE 1

Incidence of Respiratory Arrest in Unanesthetized Rabbits During Exposure to Hypoxia (5 minutes at ca. 5% O_2) Before and After Adrenergic Blocking Drugs.

Drug:	None*	Solvents*,**	Di. B.*	121	186	Pris	DHO 180***
Dose mg/Kg:			12 - 24	3-6	6	10-20	¼ - 1.0
No. Animals tested:	88	18	33	9	6	10	8
No. Animals arrested:	3	0	29	7	6	2	5

* Data previously reported (1)

** Propylene Glycol 50%, 2 cc, or Alcohol 50% average 0.5 cc, or Propylene Glycol and Absolute alcohol equal parts average 0.5 cc.

*** Rabbits in this series were subjected to recording of arterial pressure under local anesthesia Results were as follows:
MAP before drug 93 mm Hg average; MAP after drug 55 mm Hg; MAP at arrest 28 mm Hg; MAP at end hypoxia, no arrest 69 mm Hg.

ventilation did not re-establish spontaneous respiration. On the other hand, little ventilatory manipulation was ordinarily required to restore spontaneous respiration if ventilation with room air was begun within one and one half minutes of arrest. If dogs with adrenergic blockade once had respiratory arrest, there was no spontaneous return of ability to maintain respiration over a five-minute period during subsequent trials of hypoxia.

Table 2 also illustrates the fact that animals which arrested had low MAP when hypoxia was induced and underwent further fall of MAP during the period of oxygen deprivation. This was the phenomenon most constantly associated with arrest. Of 105 instances of arrest in 182 hypoxic trials in dogs only two instances occurred with MAP above 80 mm Hg at the time when breathing ceased. In 43 instances the MAP was between 50 and 79 mm of mercury. In all the rest MAP at arrest was below 50 mm of mercury. On the other hand, it must be noted that in 77 trials in which arrest did not occur, there were 10 instances where respiration continued despite MAP below 50 mm Hg. Efforts were made therefore to prevent arrest by avoiding the hypotension.

Pitressin was given by infusion to certain animals after arrest had once occurred. The solution containing 20 pressor units per 100 cc was given at a rate of 20 to 60 drops per minute initially and then reduced to 16 to 20 drops per minute. Control hypoxic trials summarized in Table 3 are from the same dogs with adrenergic blockade before Pitressin. The test hypoxia was induced while Pitressin was being given. Adrenolytic drugs used were, BBB, 186, 121, 7337.

With the same purpose in mind, animals were subjected to sub-diaphragmatic aortic occlusion. Occlusive clamps were applied at the beginning of a hypoxic trial to animals which had previously shown arrest under hypoxia

TABLE 2

Incidence of Respiratory Arrest in Anesthetized Dogs on Exposure to Hypoxia after Adrenergic Blockade Drugs and their Congeners.

Drug and (Dose mg/Kg)	Anesthetic*	% O_2 Average	# Dogs, and (# Trials) Total	With arrest	MAP After Drug	MAP at Arrest	MAP at end Hypoxia-No arrest	# with good Epinephrine reversal
None	U	8.0	58 (85)	0 (0)	135	----	150	None
None	P	7.3	22 (30)	0 (0)	120	----	137	None
NOH (6 to 12)	U	8.1	8 (28)	2 (2)	100	130, 54	113	None
184 (18)	U	8.4	6 (24)	0 (0)	105	----	113	1
Dib (12 to 24)	4P 2U	7.1	6 (28)	6 (13)	79	39	57	6
BBB (1 to 9)	U	8.2	23 (47)	23 (29)	91	52	88	20
	P	7.6	4 (15)	2 (2)	100	47	90	4
186 (6 to 12)	U	8.3	14 (28)	13 (20)	68	41	62	11 of 11 tested
121 (2 to 3)	U	9.7	6 (17)	6 (10)	67	41	65	4 of 4 tested
Pris (10 - 20)	U	6.4	6 (19)	6 (16)	91	49	80 (3 only)	6
	P	6.9	2 (9)	0 (0)	123	----	138	1 of 1 tested
7337 (2 to 5)	U	8.1	7 (19)	5 (5)	67	33	77	6
DHO (1 to 3)	U	8.3	5 (17)	1 (2)	136	32	129	None
	P	8.3	3 (9)	1 (1)	124	34	130	None

* U = Urethane
P = Pentobarbital

TABLE 3

Incidence of Arrest after Bolstering of MAP by Pitressin

Condition	Dogs (Trials)	MAP before hypoxia (mm Hg)	MAP at arrest	MAP at end hypoxia no arrest	Incidence of arrest (Trials) and %
Blockade alone	12 (31)	62	41	63	(28) 90%
Blockade with Pitressin	12 (25)	97	44	76	(11) 44%

TABLE 4

Incidence of Arrest after Bolstering of MAP by sub-Diaphragmatic Aortic Occlusion

Condition	Dogs (Trials)	MAP before hypoxia (mm Hg)	MAP Maximum during hypoxia (mm Hg)	Minimum MAP with arrest (mm Hg)	Minimum MAP no arrest (mm Hg) (Trial)	Incidence of arrest %
Blockade alone	5 (5)	93	93	61	66	(5) 100%
Blockade with aortic occlusion	5 (7)	72*	127	63	57	(4) 57%

* Note that occlusion had not been done at the time of this reading.

after BBB. Controls were done on the same animals by subjecting them to hypoxia while under adrenergic blockade, without aortic occlusion. (See Table 4). It is clear from these data: 1. The MAP pressure before hypoxia was increased by either Pitressin or aortic occlusion. 2. The total incidence of arrest was reduced by the Pitressin and by the aortic occlusion. Certain animals still arrested despite the higher pre-hypoxic pressure. Those which did arrest showed a fall of MAP to as low a level as they did without the Pitressin or occlusion.

The parallelism between good epinephrine reversal and susceptibility to respiratory arrest in the dog experiments was close if not perfect. The rabbits, of course, did not show reversal but only reduction of the pressor action of the epinephrine. (See data in footnote Table 1). Recent work (4) alleged that pilocarpine can abolish the depressor response of dogs to epinephrine after adrenergic blockade. A series was therefore undertaken in which pilocarpine was utilized in animals susceptible to arrest as detailed in Table 5. The responses of MAP to epinephrine and hypoxia are included. The pilocarpine further lowered the MAP of these dogs with adrenergic blockade. Pilocarpine reduced the depressor phase of epinephrine but did not eliminate the reversal. The incidence of arrest was not reduced by this drug. Larger doses of pilocarpine were not suggestive in their action. In a similar way atropine was tried in a few dogs because it has been claimed to abolish epinephrine reversal (5). In these experiments no effect on the reversal was noted, and the occurrence of arrest was uninfluenced.

Theoretical considerations suggested that acapnia due to hyperventilation might be responsible for the arrest. It is clear that the degree

TABLE 5

Incidence of Respiratory Arrest Before and After Attempts to Block Epinephrine Reversal with Pilocarpine.

	Before Adrenergic Blockade		After Adrenergic Blockade			
	Epinephrine	Hypoxia	Epinephrine	Hypoxia	After Pilocarpine 0.25 mg/Kg.	
					Epinephrine	Hypoxia
Dogs (Trials)	6 (9)	6 (6)	6 (6)	6 (7)	6 (7)	6 (8)
MAP before procedure	147	151	69	65	50	47
MAP at Maximum Response	208	167	53	40	43	32
MAP Change, %	+41%	+11%	-23%	-40%	-14%	-33%
Incidence of Arrest	0	0	0	6	0	7

of hyperventilation is greater in the control than in the animal with adrenergic blockade and thus the above possibility seems remote. If one postulated that the animal was rendered less sensitive to carbon dioxide by the adrenolytic agent the theory of acapneic causation would still be tenable. On this basis prevention of loss of carbon dioxide was attempted by including circa 4% carbon dioxide in the hypoxic mixture. Table 6 shows that the incidence of arrest was not reduced by carbon dioxide. The individual cases arrived at arrest on the average 1½ minutes sooner with carbon dioxide than without it. The increase in ventilation volume was not greater in the presence of carbon dioxide. Thus the occurrence of arrest would not seem to be due to acapnia.

Since stimulation of the afferent fibers in the vagus is capable of producing apnea a few trials of vagotomy were made in animals already proven subject to arrest. In no case did vagotomy prevent the expected arrest. Efforts to produce arrest by blocking motor responses of the autonomic nervous system by utilizing tetraethyl ammonium bromide in place of the adrenergic blocking agent were ineffective in reducing the resistance to hypoxia as tested by this technique. The dosage was proven adequate to block carotid occlusion pressor responses.

In order to better simulate the natural conditions of reduced oxygen tension which occur during aircraft flight, a series of 25 hypoxic trials in 17 unanesthetized dogs given potent adrenergic blocking drugs was done in an altitude chamber in ascents to 30,000 f.p.a. In this series, respiratory arrest did not occur.

Discussion: The results show that adrenergic blockade whether caused by an imidazoline derivative or by a B-chlorethylamine compound is accompanied by reduction of ability to maintain respiration in the face of hypoxic

TABLE 6

Incidence of Arrest After Attempted Prevention of Acapnia

	Before Adrenergic Blockade (BBB)					After Adrenergic Blockade (BBB)				
	Dogs (Trials)	MAP	P	MAP + P	Arrest No. (%)	Dogs (Trials)	MAP	P	MAP + P	Arrest No. (%)
8.2% O_2	6 (6)	132.5	+21.2	153.7	0	6 (6)	92.7	-31.3	66.7	5 (83.5)
4.0% CO_2 + 8.1% O_2	6 (6)	146.7	+20.3	167.0	0	6 (8)	7 9.3	-32.3	48.3	8 (100)

stress. That the impotent congeners (NOH and 184) of B-chloraethyl-amines, which are incapable of adrenergic blockade, fail to produce this decreased resistance strengthens the idea of specificity of this action. That DHO 180 does not dependably produce sensitivity to arrest may be associated with its relatively poor peripheral adrenergic blocking powers. It did not produce good epinephrine reversal in the dogs. Failure to produce arrest in the dogs might also be associated with poor hypotensive powers. In the dogs it did not lower the prehypoxic MAP. In the rabbits it produced arrest in just over half the cases and it lowered the prehypoxic MAP markedly. A corollary observation is Hazard's (6) who showed that animals poisoned with agents which interfere with autonomic transmission especially nicotine, cocaine or ergotamine die of respiratory arrest. Similarly, Du Bois and others (7,8) have emphasized that animals poisoned with cholinesterase inhibiting drugs also die of respiratory failure. Nonetheless it is indeterminate whether this type of premature respiratory arrest under hypoxia in the presence of adrenergic blockade is a specific action or a general toxic phenomenon. The arrest is not species specific since it has now been produced in rabbits, cats and dogs.

The mechanism of arrest in these animals appears to include failure of central respiratory drive. By the current postulations of respiratory physiology it would be expected that this grade of hypoxia should render the respiratory center less sensitive to carbon dioxide and the respiration would depend largely on the carotid body drive. The adrenergic blocking agents must then: 1. Either depress the output of these carotid receptors or render them more easily fatigued. The fact that hypoxia still markedly

increased the ventilation over the first minute or two favors the latter explanation.

2. Alternately the blocking agents might depress the sensitivity of the center to impulses from the carotid end organs. That the center was depressed for carbon dioxide stimulation is shown in the failure of added carbon dioxide to improve the response to hypoxia. The present data do not make the differentiation between these alternatives. It is clear that recording of potentials in Hering's nerve during arrest would be helpful. Perfusion of isolated carotids with the blocking agent and determining susceptibility to arrest might also be informative. Neither of these has been explored.

A somewhat different theory of arrest can be formulated on the basis of the concomitance of hypotension. The normal equilibration to hypoxia presumably involves substituting higher blood flow through the medullary centers for the larger arterial oxygen content presented before hypoxia. It can be postulated that the adrenergic blocking agent might prevent the increase of blood flow. This blocking of flow might result from failure of feeding head (i.e. hypotension) or from blocking of vasodilative effects in the brain. The feeding head has been demonstrated to be low. No evidence concerning blood flow or vascular reactions has been adduced. The hypoxial blood pressure response is reversed in the presence of adrenergic blockade. This might be thought of as simply a reaction to endogenous epinephrine released during hypoxia except for the fact that hypoxic reversal occurs in rabbits which do not show epinephrine reversal. Hypoxic reversal has been produced in animals by at least one other means besides adrenergic blockade. Gellhorn and Lambert (9) showed that animals in which all four of the buffer nerves had been sectioned had hypoxic reversal. Thus again interest focuses on a

possible effect of adrenergic blocking drugs on the receptors of the carotid and aortic bodies.

The rabbit was shown to be able to sustain respiration for five minutes despite adrenergic blockade if the partial pressure of oxygen was reduced slowly as in the chamber (See Bauer (10). On the contrary, if the reduction was abrupt as in the hypoxic mixture experiments the rabbit failed to maintain respiration. Since no anesthesia was involved the importance of time for adjustment tothe hypoxic stress is emphasized. In the dog experiments the factor of anesthesia eliminated the possibility of weighing directly the importance of rate of transition. Estimate of possible clinical importance of this phenomenon has to account for the likelihood that man will be asked to fly while under adrenergic blockade for therapeutic purposes. This seems much more remote in 1951 than it did in 1947.

Summary: 1. A high incidence of respiratory arrest in rabbits and dogs pretreated with various adrenergic blocking agents and subjected to hypoxia (5% to 10% O_2 in N_2 for five minutes) is demonstrated. The absence of this phenomenon in animals without drug, and in those pretreated with impotent congeners of the B-chlorethylamine derivatives is also shown. 2. The abrupt pattern of respiratory arrest previously described for the rabbits (unanesthetized) was not seen in dogs. A more gradual decrease of ventilation to arrest occurred. 3. Hypotension was observed with remarkable constancy, during the hypoxic period, in those animals which arrested. Efforts were made to prevent arrest by avoiding the hypotension. Pitressin by infusion or sub-diaphragmatic aortic occlusion raised the prehypoxic MAP of animals with adrenergic blockade. The total incidence of arrest was reduced in both test groups.

4. In the dogs a close parallelism was observed between epinephrine reversal and respiratory arrest. Pilocarpine, which has been reported to abolish the depressor phase of epinephrine reversal, was given to animals proven subject to arrest after adrenergic blockade. The incidence of arrest was not reduced. Pilocarpine further lowered MAP in animals with adrenergic blockade. The depressor phase of epinephrine reversal was reduced but the reversal was not eliminated. 5. In an effort to eliminate the influence of acapnia, 4% carbon dioxide was added to the hypoxic mixture. In a series subjected to hypoxia with the added carbon dioxide the incidence of arrest was not reduced. 6. In a series of hypoxic trials in unanesthetized dogs given potent adrenergic blocking drugs, in which hypoxia was produced by gradual ascent in an altitude chamber to 30,000 foot pressure altitude, respiratory arrest did not occur. Thus the rate of adjustment to hypoxia appears important in determining the stress tolerance of the animal with adrenergic blockade.

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Lethality of Simulated Altitude for Rabbits, Normal and Pretreated with Tetraethyl Pyrophosphate or Dibenamine

By Robert O Bauer and James Gourzis

EXPERIENCE with animals pretreated with adrenergic blocking drugs suggested a deterioration of resistance to hypoxia.[3,4] Unanesthetized rabbits, and anesthetized dogs, cats and rabbits after adrenergic blockade underwent respiratory arrest in less than five minutes when hypoxia was induced by abrupt shift from an atmosphere of room air to an atmosphere of 5 to 11 per cent oxygen. The present experiments were undertaken to learn whether poor resistance to hypoxia was demonstrated by animals with adrenergic blockade on exposure to more gradual hypoxia such as that obtained by decompression at a rate of approximately 10,000 feet per minute in an altitude chamber. Similarly, overbalance of existing adrenergic impulses was attempted in other animals by cholinergic potentiation.

Method: Albino rabbits, of both sexes, weighing between 1.8 and 3.8 Kg were used. Twelve to fifty animals were exposed in each experiment to several steps of decreasing pO_2. Hypoxia was obtained by rapid ascent in a standard portable six-man altitude chamber whose ceiling was about 45,000 feet pressure altitude (f.p.a.). The pattern of chamber flight included successive exposures at 25,000, 30,000, 35,000 and 40,000 f.p.a. The rabbits were held at each altitude for five minutes and allowed a ten-minute rest at ground level between successive hypoxic exposures. Hereafter this pattern of hypoxia shall be referred to as a serial altitude exposure. The time intervals required to reach the previously stated test altitudes were respectively: 110 ± 10 seconds, 140 ± 12 seconds, 170 ± 15 seconds, and 250 ± 20 seconds. The return to ground level was at a rate comparable to the time required for ascent. Chamber temperatures were recorded from a standard mercury thermometer before, during and after each five-minute exposure. Some animals were exposed to altitude for the first time pretreated with the drug. These are referred to as "uncontrolled" tetraethyl pyrophosphate (TEPP) or Dibenamine (DiB) injected exposure. In other cases the survivors from a control group previously exposed the same day to a serial altitude run were treated with the drug and re-exposed to hypoxia. These are called "controlled" TEPP

TABLE I. LETHALITY OF HYPOXIA FOR RABBITS WITH AND WITHOUT TEPP

Hypoxic Exposure, Drug and Dose mcg/Kg	LD50 and (CL) in thousands f.p.a.	f/LD50	S and (CL)	f/S	Number Animals	Weight and SD Kg.
First Control	38 (36.9—3[illegible] 1)	1.03	1.14	1.04	311	2.7±0.4
Second Control	40 (37.0—43.1)	1.08	1.09 (1.02—1.17)	1.07	59	2.6±0.3
TEPP—10	39 (34.8—43.7)	1.12	1.17 (1.03—1.33)	1.14	26	2.6±0.3
TEPP—50	34 (32.4—35.6)	1.05	1.11 (1.03—1.18)	1.06	59	2.5±0.3
TEPP—100	30 (?)	?	?	?	40	2.6±0.2
Controlled TEPP—10	49 (42.2—56.9)	1.16	1.4 (0.8—2.6)	1.80	54	2.6±0.3
Controlled TEPP—50	37 (35.6—38.5)	1.04	1.04 (1.03—1.11)	1.04	58	2.5±0.3
Controlled TEPP—100	?				11	

CL = 99% confidence limits. LD50 and CL in thousand f.p.a. SD = standard deviation. S = Slope. f/LD50 and f/S = factor of the respective items. For explanation of these symbols see reference 1.

or DiB injected exposure. A third group was exposed twice to altitude the same day without drugs in either case. These are referred to as "double control" exposure. Control animals were included with injected animals in each serial altitude run.

Drugs were administered ten to thirty minutes prior to the serial altitude exposure. The TEPP was diluted with absolute ethanol and DiB with propylene glycol so that the volume of the injected solution was maintained at 0.1 cc/Kg of rabbit. All dosages of TEPP were administered rapidly by the intravenous route in the marginal ear vein of the animals. DiB was administered slowly intravenously over a two-minute period. Animals injected with the respective solvent served as controls. The 40,000 f.p.a. exposure was not used with injected animals.

Preliminary intravenous LD50 dose for TEPP without hypoxia in twenty-two rabbits, calculated by the method described by Litchfield and Wilcoxon[8] was found to be 92 mcg/Kg. From this LD50 value three dosage levels were selected, 100 (LD50), 50, (LD0.01) and 10 (LD0.00) mcg/Kg. DiB dose of 6 mg/Kg was chosen on a basis of adequacy for reversal of blood pressure response to hypoxia. A dose twice this value was also used.

Results:—TEPP: The data of altitude tolerance obtained from control rabbits and from TEPP injected animals are given in Table I. It will be seen that 38,000 f.p.a. was the LD50 for albino rabbits subjected to progressive hypoxia (single control). When the survivors of the single control hypoxia were subjected to a second control serial altitude exposure the LD50 altitude ceiling increased to 40,000

TABLE II. LD50 TEPP SEA LEVEL VERSUS 35,000 F.P.A.

Hypoxic Exposure, Drug and Dose mcg/Kg	LD50 a.d. (CL) mcg/Kg	f/LD50	S and (CL)	f/S	Number Animals	Weight and SD Kg.
Sea Level	92 (61—125)	1.33	1.30 (1.12—1.51)	1.16	22	2.7±0.5
35,000 f.p.a.	29 (23—36)	1.25	3.41 (2.41—4.71)	1.38	97	2.3±0.3

CL=99% confidence limits. LD50 and CL in thousand f.p.a. SD=standard deviation. S=Slope. f/LD50 and /S=factor of the respective items. For explanation of these symbols see reference 1.

f.p.a. The 2,000 f.p.a. difference gave a P value between 0.05 and 0.04 but is not considered significant due to uncontrolled variables such as temperature.

When albino rabbits not previously exposed to hypoxia were given 10 mcg/Kg of TEPP intravenously shortly before a serial altitude exposure their hypoxic ceiling was not altered from that of a single control exposure (39,000 versus 38,000 f.p.a.) 50 mcg/Kg TEPP produced a significantly lower LD50 ceiling of 34,000 f.p.a. but the figure for 100 mcg/Kg (30,000 f.p.a.) was not statistically significant (insufficient observations.)

If rabbits were subjected to control altitude exposures and the survivors injected intravenously with TEPP twenty-four hours or longer after the control exposure the altitude ceiling was significantly (P=0.01) raised from 40,000 feet of the double control to 49,000 feet with 10 mcg/Kg and decreased significantly (P=0.01 with 50 mcg/Kg to 37,000 feet. Sufficient observations were not available to obtain a figure at 100 mcg/Kg dosage. The 9,000 foot increase in ceiling following administration of 10 mcg/Kg TEPP is of doubtful validity in spite of the calculated P value. The LD50 value was obtained by extrapolation from 35,000 feet upward. It would be of extreme interest to actually test the true value with a better pump so as to reach higher altitudes.

That the data shown in Table I were all dealing with the same mechanism in production of death from progressive hypoxia was suggested by the small slope (S) values, *e.g.* a small S value indicated a steep curve and *ergo* a narrow sharp endpoint. It was felt that if other factors entered into this mechanism the S values should deviate from parallelism. However, when TEPP was introduced into the system the S values continued to be significantly parallel to those of hypoxia alone.

In occasional animals of both control and TEPP series vigorous clonic convulsions preceded death. The incidence of this observation did not differ between the two groups. Muscular fasciculations were always observed in dying injected animals but not in the controls. On several occasions observations of heart rates of dying animals showed rates above 100 after cessation of respiration. These latter observations suggested primary central respiratory failure.

The data of Table I were recalculated to assess the LD50 dose of TEPP for animals exposed to 35,000 f.p.a. The result seen in Table II showed that TEPP at 35,000 f.p.a. was

TABLE III. LETHALITY OF HYPOXIA FOR RABBITS WITH AND WITHOUT DiB

Hypoxic Exposure, Drug and Dose mgm/Kg	LD50 and (CL) Thousand f.p.a.	f/LD50	S and (CL)	f/S	Number Animals	Weight and SD Kg.
First control	38 (36.9—39.1)	1.03	1.14 (1.10—1.18)	1.04	311	2.7±0.4
Second control	40 (43.1—37.0)	1.08	1.09 (1.02—1.17)	1.07	59	2.6±0.3
Di B—6	31.8 (30.0—33.7)	1.06	1.11 (1.05—1.18)	1.06	26	2.6±0.3
Controlled Di B—6	35.4 (32.8—38.2)	1.08	1.10 (1.02—1.19)	1.08	23	2.4±0.3
Di B—12.5	32.0 (21.3—48.0)	1.50	1.07 (N. S.)	N.S.	28	2.5±0.2

CL = 99 % confidence limits. LD50 and CL in thousand f.p.a. SD = standard deviation. S = Slope. f/LD50 and f/S = factor of the respective items. N. S. = not significant.

3.2 (2.2—4.6) times as toxic as at sea level (P=0.05).

DiB.—Table III shows that uncontrolled rabbits injected with 6 mgm/Kg of DiB and exposed to serial altitude exposures had a significantly (P=0.01) lower (6,000 feet) altitude ceiling when compared to the control values. A series of twenty-eight rabbits injected with 12.5 mgm/Kg. could not be treated statistically due to the erratic response. However, it would appear that the altitude tolerance of these latter animals is little different from those injected with 6 mgm/Kg of DiB, even though the 99 per cent confidence limits are extremely wide.

The DiB injected survivors of a controlled altitude exposure have a greater tolerance to hypoxia than do injected uncontrolled animals. Table III shows that DiB produced a 6.2 thousand foot decrease of tolerance in the uncontrolled rabbits versus 4.6 thousand foot decrease in the controlled rabbits. Taking into account the expected mortality of two serial altitude exposures the figures obtained from controlled and uncontrolled rabbits injected with 6 mgm/KgDiB retained a significant statistical difference. Slope values (S) obtained from DiB injected rabbits were not different from TEPP injected or control animals.

DiB injected rabbits dying during serial altitude exposures never convulsed. The animals became docile, respiration progressively slowed, and finally ceased.

The influence of temperature on LD50 altitude of rabbits: The rabbit data obtained from control serial altitude exposures were expressed as the LD50 altitude ceilings at various temperatures. The results were plotted against the respective mean chamber temperature (degrees centigrade) in Figure 1. Plotted in the same figure is similar data from the literature obtained by Kottke[7] using mice. The similarity of these two curves is striking especially above 25 degrees C. In general, the rabbit appeared to be less resistant to hypoxia than was the mouse. The rabbit had its greatest

hypoxia tolerance at an ambient temperature about 10 degrees C higher than the mouse although the mouse can withstand an 8,000 f.p.a. greater hypoxic stress.

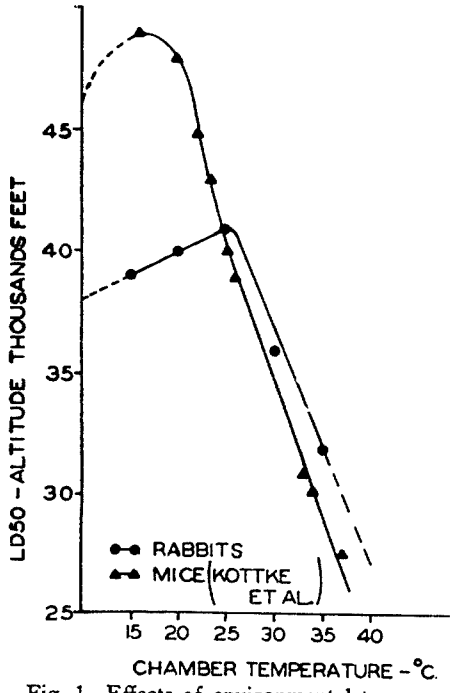

Fig. 1. Effects of environmental temperature on survival of albino rabbits subjected to progressive hypoxic stress. Each point represents the LD50 altitude ceiling for a given temperature.

Discussion.—The graded decrease in simulated altitude tolerance with increasing doses of TEPP suggests progressive depression of the rabbits' normal respiratory stability. Sublethal doses of TEPP are known to severely inhibit both peripheral and central cholinesterase (ChE).[2,5,6] Whether this ChE poison produced its effect in these experiments by peripheral or central nervous system ChE depression was not demonstrated. The absence of difference in mortality slope values between TEPP, DiB and control hypoxic exposures is remarkable especially since the agents are so different in chemical composition and physiologic action.

It is of interest to consider whether these two agents (TEPP and DiB) or similar chemical analogues would effect altitude tolerance adversely when used in men subjected to hypoxic stress of routine flight in standard civilian or military aircraft. It may be that the reduction of altitude tolerance by these drugs would not appear with the slower ascent rates of conventional flight. Drug dosage in the rabbits was well above safe clinical limits. Human dosage of these materials would not be likely to restrict the compensatory mechanisms ordinarily operating during hypoxic stress. It is hard to visualize clinical usefulness of either agent *per se*. New and clinically useful analogues may well be described in the future. Dubois[1] recently described a TEPP analogue, Octamethyl pyrophosphoramide (OMPA), which appears to offer success in the treatment of myasthenia gravis. It would be of interest to know whether Neostigmine limits the altitude tolerance of myasthenic patients.

SUMMARY

1. The altitude tolerance of albino rabbits has been measured by serial five minute exposure to pressure altitudes of 25,000, 30,000, 35,000 and 40,000 feet successively. Ten-minute rest at ground level was provided between the successive exposures of each series.

2. The LD50 pressure altitude for albino rabbits on first exposure proved to be 38,000 feet. Survivors of such a first series demonstrated a statistically insignificant 2,000 foot increase

in ceiling (LD50) on subsequent exposure.

3. Environmental temperature was shown to have a significant effect on altitude tolerance of rabbits. Maximum tolerance for altitude was obtained at 25° C. ambient temperature.

4. Disruption of the parasympathetic-sympathetic balance of these animals by prior treatment with dibenamine (adrenergic blockade) or Tetraethylpyrophosphate (cholinergic potentiation) reduced the altitude tolerance significantly.

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